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THE EFFECT OF DIBUTYL- AND DIOCTYLPHTHALATES ON RATS WITH ORAL FEEDING DURING PROLONGED TESTING. PART I. APPLICATION AND TOXICOLOGICAL PROPERTIES OF DIBUTYL- AND DIOCTYL-PHTHALATES

Hanna Piekacz

National Institutes of Health

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22:55-61, 1971THE EFFECT OF DIBUTYL- AND DIOCTYL-PHTHALATES ON RATS
WITH ORAL FEEDING DURING PROLONGED TESTING

by Hanna Piekacz

Part 1. Application and Toxicological Properties
of Dibutyl- and Dioctyl-Phthalates

From a research report on foodstuffs and items of State use by the Institute of Hygiene, Warsaw (Prof. M. Nikonorow, Director).

The use of dibutyl- and dioctyl-phthalates is discussed a propos of legislation in various countries, as well as their toxicological properties. This study serves as an introduction to research on hyperacute and extended toxicity of both phthalates.

Growth in the manufacture of polyvinyl-chloride, as well as the trend toward using it in items which come in contact with food and human beings, has elicited the need to evaluate the dangers inherent in this material.

Polyvinyl chloride is a macromolecular compound, which in itself is of no danger to health security. A health problem does arise, however, as a result of supplementary chemicals used in its manufacture.

Of all the supplementary chemicals which are added to polyvinyl-chloride, the mollients are of prime importance. They are solids or oily liquids, typified by their low vapor pressure and high boiling point. These mollients are added to the material in quantities which correspond to the physical properties and the chemical stability of the manufacture item.

Mollients impart ductility and plasticity to the material, likewise enhancing its fluidity during molding. The presence of mollients causes a reduction in molding temperature, which also facilitates that method of processing. This is rather significant in polyvinyl-chlorides having low mollient content (less than 10%).

Polyvinyl-chloride may be produced "soft" or "hard", according to the percentage of mollients added. Those products which are almost free of mollients are called *hard polyvinyl-chloride*. These items are often called by the name "vinyl-dur". Products containing 10% or more mollient are called *soft polyvinyl-chloride*.

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ide (mollients sometimes constitute as much as 60% of the basic material). Products which are semi-hard or soft may also be plastic. These are often grouped under the term "Igelite".

In most compounds the mollients are micromolecular, readily soluble in water, fat, and oil, and may consequently migrate from the material to the foodstuffs coming in contact with the polyvinyl-chloride (particularly foods containing fats) [1-11]. At the same time, these mollients facilitate absorption of supplementary chemicals into the food, and these are often quite toxic stabilizers [12-16].

In Poland, polyvinyl-chloride is manufactured using dibutyl- $[C^6H^4(COOC^4H^9)^2]$ and dioctyl- $[C^6H^4(COOC^8H^{17})^2]$ phthalates. Both compounds have been the objects of personal research of the foreign authors. Their findings, however, are not identical, which is a reflection of differing legislation in many countries.

Dibutyl-phthalate is not allowed in the manufacture of polyvinyl-chloride items which contact with food in the Soviet Union [17], Czechoslovakia [18], and DDR [19], among others. This compound also does not appear in the list of permitted additives used in the manufacture of artificial materials for the aforementioned trade purposes in the United States [20], Denmark [21], Latin America [22], and Poland [23]. In France, however, in 1959 it was introduced into an order on packaged articles intended for direct contact with foodstuffs, at 20 parts phthalate per 100 parts polyvinyl-chloride (20%).

West Germany also permits the use of dibutyl-phthalate to soften PVC intended for the manufacture of some items, e.g., tubes and conveyor belts, at 40%, noting a low degree of migration to food during contact with the conveyor belt of less than one minute [25]. Moreover, West Germany also permits this compound to be added to cellophane at 5 mg/dcm² [26] and at 10 mg/dcm² [27]; to plexiglas it may be added up to 3% [28].

In accordance with instructions from the authorities in that country, softened PVC may not be used in items coming into contact with free-flowing foodstuffs (cereal, flour), or those containing fat, those which are paraffined or waxed, milk or milk-byproducts, or foods containing alcohol or essential oils.

Dibutyl-phthalate also appears in the list of supplementary substances used in the manufacture of PVC in Italy [29].

Dioctyl-phthalate may be used as an additive to PVC intended for contact with foodstuffs in many countries [17-25, 29]; some countries restrict its use for fats and food containing fats [20, 21, 23, 25, 29].

A study of dibutyl- and dioctyl-phthalates has shown that they are compounds having low toxicity [30, 31].

TOXICOLOGICAL RESEARCH IN THE LIGHT OF LITERATURE ON
ON DIOCTYL-PHTHALATE
(aka 2-diethylohexyl-phthalate [32-35]).

Hodge [36], in studying acute toxicity by feeding rats and mice (by mouth and through peritoneum) with dioctyl-phthalate asserted its comparatively low degree of toxicity. This has also been confirmed by the research of other authors [35, 37-39]. Mallette and von Hamm [40], in experiments on rats, discovered that feeding them a dose in excess of 2g/kg body weight caused their death. Lower doses, however, caused a drop in body weight, leucocytosis, and acute anemia. The animals regained their health after 1-2 months. Authors have also asserted a moderate stimulatory effect of that compound on the skin.

Carpenter et al [41] added dioctyl-phthalate to the diet of rats for a period of 2 years in dosages of 6.4; 0.13; and 0.04%. With doses in excess of 0.4%, they observed a significant decrease in body weight, and also a statistically significant increase in the average weight of the liver and kidneys. In a one-year study by these authors on guinea pigs and dogs, they proved that when the aforementioned compound was administered in doses of 0.06 ml/kg body weight, there was no injurious effect on the animals.

In studying the extended toxicity of dioctyl-phthalate [32], added to a rat's diet for 21 months in doses of 250 and 500 mg (the experiment was carried out for four generations at those dosages) and 2.0 g/kg, there was no indication of change in the growth curve of the animals studied (in comparison with a control group) or anomaly in the reproduction of the animals. The experiment also indicated the absence of carcinogenic properties in the chemical.

Halpern and Weiss [42] tested a "fringe" group of 200 volunteers, using a vinyl sheet containing dioctyl phthalate as a mollient. The authors did not assert any kind of irritation or allergic reaction. They did feel, however, that the use of that mollient in manufacturing PVC sheets for packaging foodstuffs called for further research.

Shaffer et al [37], in studying the metabolism of dioctyl-phthalate, asserted the presence of free phthalic acid in the

urine of rabbits. The authors are convinced that the ester is lost during hydrolysis. They further assert that the harmful effect of the ester is totally dependent on the radical and consequently, on the parts of alcohol molecules.

Hodge [36] proved that 2-diethylohexyl ester is tenfold less toxic than alcohol. The low toxicity of phthalic acid esters suggests [43] that *in vivo* they do not decompose to form highly toxic acid and alcohol. Erickson confirmed this [44] in a study on dogs, showing that 92-94% of the diphenyl-phthalate and 97-91% of the butyl-benzyl-phthalate doses administered were excreted in an unchanged form.

Nevertheless, Smith [45], in an experiment conducted *in vitro*, showed that dibutyl-phthalate may decompose by means of pancreatic lipase. This may thus reveal a definite difference in the behavior of aromatic and aliphatic esters.

Shaffer et al [37], in administering to rats per os, determined that LD50 for o-phthalic acid constituted 7.9 g/kg; for diethylohexanol - 7.1 g/kg, whereas for dioctyl-phthalate - 30.6 g/kg body weight. None of these studies has proven any anatomic/pathological changes in the organs of the animals being tested.

Frawley [46] observed that a dose of 2-diethylohexyl-phthalate, administered in the amount of 1300 mg/kg for a 2-year feeding period, had no toxic effect on the animals tested (so-called "no-effect level").

McLaughlin et al [47] confirmed these results. The above compound, tested in a dose of 2000 mg/kg on chicken embryos, had no deleterious effect.

In accordance with the directives of the FAO/WHO [48], the allowable daily intake (ADI) of dioctyl-phthalate for humans can constitute up to 1.0 mg/kg body weight, conventionally 1-2 mg/kg.

Lehman [49, 50] stressed that PVC sheets softened with dioctyl-phthalate may only be used in food packaging where there is a high content of water; it may not be used, however, where there is a fat content, or with food containing fats.

A number of completed studies administered the above mollient by other methods [31, 51]. A 250 mg/kg b.w. dose of dioctyl-phthalate was injected peritoneally into mice for 6 weeks, observing a retardation in body-weight increase and the onset of

peritonitis. Experiments conducted on mouse fibroblasts [51] showed no toxic effect on the cells from the compound.

Haberman et al [52] observed a definite teratogenic effect of dioctyl-phthalate upon administering that compound to the fibro-urinary membrane of 7-day-old chick embryos. Out of a total of 32 hatched eggs, one bird had no left eye and had a deformed lower jaw.

DIBUTYL-PHTHALATE

The research of Smith [45] and also Wilson and Cormick [53] proved the low toxicity of dibutyl-phthalate. During oral and intramuscular administration, the authors of [53] established $LD_{50} = 8 \text{ g/kg}$. Hodge et al [54], in administering the compound to mice via peritoneum gave $LD_{50} = 3.5 \text{ ml/kg}$ (3.7 g/kg), whereas Karel et al [55] - 3.96 ml/kg (4.15 g/kg).

Experiments conducted on extended toxicity in rats confirmed the weak toxicity of the aforementioned mollient [32]. The compound in question was administered - by calculating it per kg of diet - in 100 mgs to 5 generations and 300 and 500 to three generations. Separately, the rats received the above phthalate in doses of 100 and 300 mg/kg of their diet for 21 months and 500 mg/kg of their diet for 15 months.

The resulting findings, under the conditions of the experiment conducted, permitted a conclusion on the absence of deleterious effects from dibutyl-phthalate, chiefly the absence of carcinogenic properties in the chemical. Justification was manifested in the animals' reproduction. It is significant that the dose used corresponds to a daily ration of dibutyl-phthalate to a man weighing 70 kg of average activity (daily ration 2500 calories per day).

Bornmann et al [56], in a year-long study on rats, administered dibutyl-phthalate diluted 1:1 with oil, in doses of 0.5 and 1.0 g/kg body weight, orally two times per week; he found three cases of onset of changes in neoplasm of the sarcoma type. In this aspect, the authors are looking for further conclusive studies to determine whether the mollient in question has a carcinogenic effect.

The research of Homrowski and Nikonow [57], concerned with determining the average fatal dose for both mollients, dibutyl- and dioctyl-phthalate (made in Poland), with administration by mouth and peritoneally to rats, proved the amount of LD_{50} to be



low for dibutyl-phthalate.

The finding of these authors is shown in Table I.

Table I
ACUTE TOXICITY OF DIBUTYL- AND DIOCTYL-PHTHALATES
DETERMINED BY THE KÄRBER METHOD ACCORDING TO
HOMROWSKI AND NIKONOROW [57]

Compound	Observation Period	Method of Administration	Toxicity LD50
Dibutyl-phthalate	24 hrs	oral	13.8 ml/kg
		peritoneal	8.3 ml/kg
	7 days	oral	11.9 ml/kg
		peritoneal	6.8 ml/kg
Dioctyl-phthalate	24 hrs	oral	45.1 ml/kg
		peritoneal	50.5 ml/kg
	7 days	oral	33.7 ml/kg
		peritoneal	37.3 ml/kg

All animals studied became heavy, manifested uncertainty of limb, diarrhea, and overall poor condition. Animals to which dibutyl-phthalate was given had bristly, dull hair; however, rats that received 2-diethylhexyl-phthalate had wet, sticky hair, probably as a result of the intense excretion of the chemical through the skin. In small doses, the symptoms of poisoning were rather poorly visible, and after 4-5 days (after cessation of administration of chemical), these symptoms vanished.

In microscopic preparations of liver taken from animals which had received dibutyl-phthalate, there were changes of a regressive nature, e.g., darkening of flesh, degeneration of vacuoles and, in individual cases, probably, degeneration of fat. In others, in addition to the changes indicated, there were definite cases of irritation to the endothelial reticular system. Moreover, there was also necrosis of liver flesh. The width and number of necrotic niduses varied within a wide range.

Radeva and Dincova [58], administering dibutyl-phthalate daily in rats' diet, in quantities 0.1; 1.0 and 10.0 mg/kg body weight for 7 months, did not observe an accumulation of the compound in question nor any catalytic activity. The magnitude of the admitted daily intake (ADI) of dibutyl-phthalate has been accepted as being identical (for humans) with that of

dioctyl-phthalate [48].

Wilson and Cormick [53] conclude, on the basis of experiments *in vitro*, that dibutyl-phthalate is metabolized in the same way as fat produced normally in the diet.

Smith [45] proposes that the compound is hydrolyzed through pancreatic lipase *in vitro* as rapidly as is glycerol trioleate. He affirms the thesis of the first two authors, that mollients are probably transported through the system in the same way as fats, and are also metabolized in the same way.

Wilson and Cormick [53] do not suggest the safe use of PVC sheets containing this mollient for packaging foodstuffs. This point of view is shared also by Lehman [49], who suggested that dibutyl-phthalate in non-allowable plasticizer sheets be used for softening sheets intended for contact with foodstuffs.

Among many authors, the view unanimously prevails that the degree of toxicity of phthalic acid esters is closely linked with their solubility and molecular weight [59]. Esters with high solubility and low molecular weight manifest the strongest toxic effect.

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